IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney Docket No.:	**DEX-0087**
Inventors:	**Recipon et al.**
Serial No.:	**09/705,500**
Filing Date:	**November 3, 2000**
Examiner:	**Canella, Karen A.**
Group Art Unit:	**1642**
Title:	**A Novel Method of Diagnosing, Monitoring, Staging, Imaging and Treating Cancer**

DECLARATION UNDER RULE § 1.131

I, Roberto A. Macina, hereby declare:

1. I was awarded a M.S in Biology, and a Ph.D. in 1990 in Molecular Biology from the University of Buenos Aires, Argentina. After obtaining these degrees, I spent four years at The Wistar Institute, University of Pennsylvania contributing to the Human Genome Project endeavor. From 1995 to 1997, I served in the Molecular Diagnostic Department at SmithKline Beecham holding the positions of Investigator and Senior Investigator. Since the inception of diaDexus, Inc. in 1997 I have served as the Assistant Director of Cancer Gene Discovery. In October 2001 I assumed the position of Director of Molecular Technologies at diaDexus, Inc.

2. As the Director of Molecular Technologies for diaDexus, Inc., and a named inventor, I am familiar with the teachings of the above-referenced patent application.

3. The use of Lng108, also known as fy108, as a cancer diagnostic was conceived and reduced to practice at diaDexus prior to October 27, 1999.

4. Specifically, Lng108 relative expression was determined in accordance with our standard Quantitative Polymerase Chain Reaction (QPCR) protocol and outlined in the above-referenced patent application at page 20, line 16 through page 21, line 13; and page 17, line 12 through page 18, line 9 of priority application U.S. Provisional No. 60/163,444, filed on November 4, 1999.

5. I personally supervised QPCR experiments as described in the patent application above and herein, to measure the relative levels of Lng108 in cancerous, normal-adjacent, and normal tissues. In these experiments and analyses performed prior to October 27, 1999, relative quantitation of gene expression was done using Polymerase Chain Reaction in real time.

Real-Time quantitative PCR with fluorescent Taqman probes is a quantitation detection system utilizing the 5′-3′ nuclease activity of Taq DNA polymerase. The method uses an internal fluorescent oligonucleotide probe (Taqman) labeled with a 5′ reporter dye and a downstream, 3′ quencher dye. During PCR, the 5′-3′ nuclease activity of Taq DNA polymerase releases the reporter, whose fluorescence can then be detected by the laser detector of the Model 7700 Sequence Detection System (PE Applied Biosystems, Foster City, CA, USA).

Amplification of an endogenous control was used to standardize the amount of sample RNA added to the reaction and normalize for Reverse Transcriptase (RT) efficiency. Either cyclophilin, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) or 18S ribosomal RNA (rRNA) was used as this endogenous control. To calculate relative quantitation between all the samples studied, the target RNA levels for one sample were used as the basis for comparative results (calibrator). Quantitation relative to the calibrator can be obtained using the standard curve method or the comparative method (User Bulletin #2: ABI PRISM 7700 Sequence Detection System).

The tissue distribution, and the level of the target gene for every example in normal and cancer tissue were determined. Total RNA was extracted from normal tissues, cancer tissues, and from cancers and the corresponding matched adjacent tissues. Subsequently, first strand cDNA was prepared with reverse transcriptase and the polymerase chain reaction was done using primers and Taqman probe specific to each target gene. The results are analyzed using the ABI PRISM 7700 Sequence Detector. The absolute numbers are relative levels of expression of the target gene in a particular tissue compared to the calibrator tissue.

6. Attached hereto are copies of laboratory notebook pages 0034-124, 0034-168 and 0049-048 containing QPCR experimental procedures and results for Lng108, also known as fy108. The actual dates, all prior to October 27, 1999, have been redacted from the attached copies. These experiments demonstrated the use of Lng108 as diagnostic marker for cancer. All these experiments were performed under my direct supervision prior to October 27, 1999.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

01/08/2004
Date

Roberto A. Macina